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## Limited Sampling Strategies Using Linear Regression and the Bayesian Approach for Therapeutic Drug Monitoring of Moxifloxacin in Tuberculosis Patients

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- 1    **Limited sampling strategies using linear regression and the Bayesian approach for therapeutic drug**  
2    **monitoring of moxifloxacin in tuberculosis patients**  
3  
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32

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36

37 **Abstract**

38

39 Therapeutic drug monitoring (TDM) of moxifloxacin is recommended to improve response to  
40 tuberculosis treatment and reduce acquired drug resistance. Limited sampling strategies (LSSs) are able  
41 to reduce the burden of TDM by using a small number of appropriately timed samples to estimate the  
42 parameter of interest; the area under the concentration time curve. This study aimed to develop LSSs for  
43 moxifloxacin alone (MFX) and together with rifampicin (MFX+RIF) in TB patients.

44 Population pharmacokinetic (popPK) models were developed for MFX (n=77) and MFX+RIF (n=24).

45 Additionally, LSSs using Bayesian approach and multiple linear regression were developed. Jackknife  
46 analysis was used for internal validation of the popPK models and multiple linear regression LSSs.

47 Clinically feasible LSSs (1-3 samples; 6 h timespan post-dose; 1 h interval) were tested.

48 Moxifloxacin exposure was slightly underestimated in the one compartment models of MFX (mean -  
49 5.1%, standard error [SE] 0.8%) and MFX+RIF (mean -10%, SE 2.5%). The Bayesian LSSs for MFX and  
50 MFX+RIF (both 0 and 6 h) slightly underestimated drug exposure (MFX mean -4.8%, SE 1.3%; MFX+RIF  
51 mean -5.5%, SE 3.1%). The multiple linear regression LSS for MFX (0 and 4 h) and MFX+RIF (1 and 6 h),  
52 showed a mean overestimation of 0.2% (SE 1.3%) and 0.9% (SE 2.1%), respectively.

53 LSSs were successfully developed using the Bayesian approach (MFX and MFX+RIF; 0 and 6 h) and  
54 multiple linear regression (MFX 0 and 4 h, MFX+RIF 1 and 6 h). These LSSs can be implemented in clinical  
55 practice to facilitate TDM of moxifloxacin in TB patients.

## 56 Introduction

57 Each year, the global tuberculosis (TB) incidence declines with approximately 2%, while by 2020 an  
58 annual 4-5% decline is strived for by the World Health Organization (WHO).(1) Multidrug-resistant TB  
59 (MDR-TB) remains a major problem with an estimated number of 458,000 cases in 2017.(1) Currently,  
60 the worldwide success rate of MDR-TB treatment is 55% and this is considered low when compared to a  
61 success rate of 85% for drug-susceptible TB (DS-TB).(1)

62 Moxifloxacin, a fluoroquinolone, is one of the most important drugs for the treatment of MDR-TB(2), but  
63 has also been used as an alternative to first-line anti-TB drugs if not well tolerated or suggested to  
64 include in case of isoniazid resistance.(3–5) In general, the toxicity profile of moxifloxacin is rather mild,  
65 though it includes concentration dependent QTc interval prolongation and, rarely, tendinopathy.(6–9) A  
66 clinically relevant drug-drug interaction is the combination of moxifloxacin with rifampicin, since these  
67 two drugs can be used concomitantly in TB treatment. Rifampicin lowers the moxifloxacin area under the  
68 concentration-time curve of 0-24 h ( $AUC_{0-24}$ ) with approximately 30% by inducing phase II metabolising  
69 enzymes (glucuronosyltransferase and sulphotransferase).(10–12)

70 The efficacy of fluoroquinolones is related to the ratio of  $AUC_{0-24}$  to minimal inhibitory concentration  
71 ( $AUC_{0-24}/MIC$ ).(13, 14) The fluoroquinolone exposure is effective against gram-negative bacteria at an  
72  $AUC_{0-24}/MIC > 100-125$  and against gram-positive species at an  $AUC_{0-24}/MIC > 25-30$ .(13, 15, 16) An *in vitro*  
73 moxifloxacin exposure of unbound ( $f$ ) $AUC_{0-24}/MIC$  of  $> 53$  was able to substantially decrease the total  
74 population of *M. tuberculosis* with over  $3 \log_{10}$  CFU/ml as well as suppress emergence of drug resistance,  
75 while an  $fAUC_{0-24}/MIC > 102$  completely killed the fluoroquinolone sensitive population of *M. tuberculosis*  
76 without observing development of drug resistance.(17) Approximately 50% of moxifloxacin is assumed to  
77 be protein bound, although protein binding is highly variable between individuals and might be  
78 concentration dependent.(13, 16, 18, 19) Corresponding with  $fAUC_{0-24}/MIC > 53$  and a fraction unbound  
79 of 0.5, the target total (bound and unbound)  $AUC_{0-24}/MIC > 100-125$  is regularly used in TB, because

individual data of protein binding is often lacking.(18, 20, 21) In case of a proven susceptibility for  
moxifloxacin while lacking a MIC value of the strain, the target  $AUC_{0-24}$  is generally set at >50-65 mg·h/L  
based on a critical concentration of 0.5 mg/L.(22, 23)

Therapeutic drug monitoring (TDM) is recommended by the American Thoracic Society for all second-line  
drugs, including moxifloxacin.(24, 25) It is important to monitor the moxifloxacin exposure in TB patients  
to determine an individualized dose, because of substantial inter-individual pharmacokinetic variability  
and relevant drug-drug interactions with the risk of treatment failure and developing drug resistance.(18,  
26–28) However, routine TDM to estimate  $AUC_{0-24}$  requiring frequent blood sampling is time-consuming,  
a burden for patients and health care professionals, and expensive. Optimising the sampling schedule by  
developing a limited sampling strategy (LSS) could overcome these difficulties with TDM in TB  
treatment.(29)

There are two main methods to develop a LSS; the Bayesian approach and multiple linear regression.(30)

The advantages of the Bayesian approach are the flexible timing of samples as the population  
pharmacokinetic model can correct for deviations and that it takes a number of parameters into account  
for example sex, age, and kidney function, leading to a more accurate estimation of  $AUC_{0-24}$ . The  
advantage of multiple linear regression-based LSSs is that these do not require modelling software and  
 $AUC_{0-24}$  can be easily estimated using only an equation and the measurement of drug concentrations.

The disadvantage is that samples must be taken exactly according to the predefined schedule and the  
population of interest should be comparable because patient characteristics are not included in the  
equations to estimate drug exposure.(30)

Pranger *et al* described a LSS for moxifloxacin for the first time using  $t=4$  and 14 h post-dose samples.(21)

This sampling strategy can be considered unpractical to be used in daily practice. Magis-Escurra *et al*  
described LSSs to simultaneously estimate  $AUC_{0-24}$  of all first-line drugs together with moxifloxacin ( $t=1$ ,  
4, 6 h or  $t=2$ , 4, 6 h), but did not differentiate between patients using moxifloxacin alone and

104 moxifloxacin in combination with rifampicin.(20) Therefore the influence of the drug-drug interaction  
105 between moxifloxacin and rifampicin, namely an increased moxifloxacin clearance, was not taken into  
106 account in these LSSs.  
107 Therefore, the aim of this study was to develop and validate two population pharmacokinetic models of  
108 moxifloxacin (alone and with rifampicin) along with clinically feasible LSSs using the Bayesian approach  
109 as well as multiple linear regression for the purpose of TDM of moxifloxacin in TB patients.

110

111

## 112 Results

### 113 Study population

114 The group with moxifloxacin alone (MFX) included pharmacokinetic profiles of 77 TB patients and the  
115 group with moxifloxacin together with rifampicin (MFX+RIF) included profiles of 24 TB patients (Figure  
116 1). The baseline characteristics sex, age and height were significantly different ( $P<0.05$ ) between these  
117 two groups (Table 1). Additionally, the  $AUC_{0-24}$  calculated with the trapezoidal rule ( $AUC_{0-24, ref}$ ) was  
118 significantly lower and time of peak concentration ( $T_{max}$ ) was significantly earlier in the MFX+RIF group  
119 ( $P<0.05$ , Table 2). Several abnormal pharmacokinetic curves (e.g. delayed absorption or single aberrant  
120 data point) were observed in both the MFX and MFX+RIF group.

121

### 122 Population pharmacokinetic model

123 For both MFX and MFX+RIF, an one compartment model with lag time resulted in the lowest Akaike  
124 Information Criterion (AIC) values and described the data best (Table 3). Two compartment models were  
125 not favourable for either MFX or MFX+RIF. A statistical comparison of the pharmacokinetic parameters  
126 of the MFX versus MFX+RIF model was provided in Table 4. Total body clearance (CL) was higher and lag  
127 time ( $T_{lag}$ ) was shorter in the MFX+RIF model ( $P<0.05$ ). Internal validation of the two models resulted in a

128 mean underestimation of  $AUC_{0-24}$  of 5.1% (standard error (SE) 0.8%) in the MFX model and a mean  
129 underestimation of 10% (SE 2.5%) in the MFX+RIF model (Figure 2A and Figure 3A). In the validation of  
130 the MFX model, an  $r^2$  of 0.98, y-axis intercept of -0.3 (95% CI -1.1 to 0.5), and slope of 0.96 (95% CI 0.94-  
131 0.98) was found in the Passing Bablok regression (Figure 2B). For the MFX+RIF model, an  $r^2$  of 0.94, y-axis  
132 intercept of -1.0 (95% CI -4.1 to 0.9), and slope of 0.98 (95% CI 0.92-1.07) was found in the Passing  
133 Bablok regression (Figure 3B).

134

135 LSS using the Bayesian approach

136 The best performing LSSs of MFX and MFX+RIF are shown in Table 5 and Table 6, including mean  
137 prediction error (MPE), root mean squared error (RMSE), and  $r^2$  to evaluate the performance of the LSSs.

138 The performance of the LSS using t=2 and 6 h samples was evaluated as well, because this strategy is  
139 currently used in many health facilities for TDM of anti-TB drugs.(31) Not all strategies met the pre-set  
140 acceptance criteria (RMSE<15%, MPE<5%).(21) Low  $r^2$  values were observed which were caused by high  
141 interindividual variability in performance of the LSSs.

142 For the MFX model, an LSS using t=0 and 6 h samples was chosen for further evaluation (RSME=15.17%,  
143 MPE= 2.42%,  $r^2$ =0.874), because it required one sample less than the three-sample strategies, while  
144 RMSE was only slightly above 15%. The internal validation showed a mean underestimation of 4.8% (SE  
145 1.3%). However, low  $AUC_{0-24}$  values were more frequently overestimated in contrast to  $AUC_{0-24} > 40$   
146 mg\*h/L mainly being underestimated by the LSS (Figure 4A). The Passing Bablok regression showed an  $r^2$   
147 of 0.94, y-axis intercept of 3.4 (95% CI 1.6-4.9), and slope of 0.85 (95% CI 0.80-0.91) (Figure 4B).

148 For the MFX+RIF model, an LSS using t=0 h and 6 h samples was chosen for further evaluation  
149 (RSME=15.81%, MPE= 2.35%,  $r^2$ =0.885), because of the benefit of requiring only 2 samples while  
150 performance in terms of RSME and MPE remained acceptable. The internal validation showed a mean



underestimation of 5.5% (SE 3.1%) in the Bland-Altman plot and an  $r^2$  of 0.90, y-axis intercept of -1.3 (95% CI -4.4 to 2.8), and slope of 1.0 (95% CI 0.88-1.10) in the Passing Bablok regression (Figure 5).

LSS using multiple linear regression

Table 7 and Table 8 show the best performing LSSs for MFX and MFX+RIF. The performance of the frequently used LSS using t=2 and 6 h samples was evaluated as well and included in the tables. None of the MFX LSSs met the acceptance criteria (RMSE<15%, MPE<5%) as bias was above 5% for all combinations. For MFX+RIF, the two three-sample strategies and LSS using t=1 and 6 h samples met the acceptance criteria.

The MFX LSS using t=0 and 4 h samples (RSME=9.25%, MPE= 6.85%,  $r^2$ =0.957) had a comparable performance to the three-sample strategies while being more clinically feasible and therefore was chosen for further evaluation. In contrast to the Bayesian LSSs for MFX and MFX+RIF, a t=0 and 6 h strategy was not feasible using a multiple linear regression approach as its performance was substantially worse (RMSE=12.01, MPE=9.43,  $r^2$ =0.905) than the LSS using t=0 and 4 h samples. Internal validation of this t=0 and 4 h LSS for MFX showed a mean overestimation of 0.2% (SE 1.3%) in the Bland-Altman plot and an  $r^2$  of 0.95, y-axis intercept of 0.1 (95% CI -2.1 to 1.6), and slope of 0.99 (95% CI 0.95-1.06) in the Passing Bablok regression (Figure 6).

For MFX+RIF, the LSS using t=1 and 6 h samples (RSME=6.09%, MPE= 4.83%,  $r^2$ =0.971) was chosen for further evaluation, because of clinical suitability in addition to good performance (RMSE<15%, MPE<5%). Internal validation showed a mean overestimation of 0.9% (SE 2.1%) in the Bland-Altman plot and an  $r^2$  of 0.96, y-axis intercept of -0.2 (95% CI -4.9 to 2.3), and slope of 1.02 (95% CI 0.88-1.15) in the Passing Bablok regression (Figure 7).

## Discussion

175 In this study, we successfully developed a population pharmacokinetic model for moxifloxacin alone and  
176 in combination with rifampicin. Furthermore, we developed and validated sampling strategies using the  
177 Bayesian approach (MFX and MFX+RIF t=0 and 6 h) and multiple linear regression (MFX t=0 and 4 h;  
178 MFX+RIF t=1 and 6 h) for both groups as well.

179 It was decided to develop two separate population pharmacokinetic models, and therefore also separate  
180 LSSs, for moxifloxacin alone and in combination with rifampicin after observing a significant effect of  
181 rifampicin on the pharmacokinetics of moxifloxacin. The population pharmacokinetic model of MFX+RIF  
182 showed an approximately 35% higher total body clearance of moxifloxacin when compared to the MFX  
183 pharmacokinetic model (Table 4). This was to be expected as rifampicin enhances metabolism of  
184 moxifloxacin and increases in total body clearance of 45-50% have been reported by others.(10, 32) As a  
185 result of this drug-drug interaction, pharmacokinetic profiles of MFX+RIF showed reduced moxifloxacin  
186 concentrations and 25% lower median moxifloxacin  $AUC_{0-24}$  values after administration of a similar dose  
187 (Figure 1, Table 2). The latter is confirmed by a significant -17% difference in dose-corrected  $AUC_{0-24,ref}$   
188 between the MFX and MFX+RIF group (Table 2). The decrease in moxifloxacin exposure by rifampicin was  
189 estimated at 30% in previous studies (10, 12, 32), although others found non-significant or smaller  
190 decreases in moxifloxacin  $AUC_{0-24}$ .(21, 33) In this study we observed only a slightly smaller effect of  
191 rifampicin on the total body clearance and exposure than previously reported. This might be explained  
192 by the possibility that maximal enzyme induction was not achieved yet at the moment of sampling in a  
193 few cases, since it generally takes around 10-14 days of rifampicin treatment to reach maximal  
194 induction.(34) Furthermore, we encountered a significant, but small, difference in lag time between the  
195 MFX and MFX+RIF models and in  $T_{max}$  of the included pharmacokinetic profiles. The faster absorption of  
196 moxifloxacin in combination rifampicin was found in other studies as well, however some reported the  
197 opposite effect. This could suggest that lag time and  $T_{max}$  was not influenced by rifampicin, but more

likely by other differences between the MFX and MFX+RIF group such as concomitantly taken TB drugs or inter-individual differences in absorption due to disease state.

In addition to the population pharmacokinetic models, we developed and validated LSSs using the Bayesian approach as well as multiple linear regression for MFX and MFX+RIF. LSSs of moxifloxacin have been described before. Pranger *et al* found a Bayesian LSS with a comparable performance (RMSE=15%, MPE=-1.5%,  $r^2=0.90$ ) when compared to our LSSs for MFX and MFX+RIF.(21) The LSS of Magis-Escurra *et al* performed better (RMSE=1.45%, MPE=0.58%,  $r^2=0.9935$ ) than the multiple linear regression LSSs proposed in this study.(20) However, a smaller sample size (n=12) was used to establish the equation and this was not externally validated. Further, we provided suitable sampling strategies for multiple situations; in patients using moxifloxacin alone or together with rifampicin and for centres that either do or do not have pharmacokinetic modelling software available. Health care professionals may select the LSS that is the most applicable to the circumstances.

The Bayesian LSS for MFX (t=0 and 6 h) showed a slight downward trend between the bias of the estimated AUC<sub>0-24</sub> and the mean of the estimated and actual AUC<sub>0-24</sub> (Figure 4). Low AUC<sub>0-24</sub> values were more frequently overestimated in comparison to higher AUC<sub>0-24</sub> values. A possible cause might be that we could not differentiate between metabolic clearance and renal clearance in both population pharmacokinetic models due to a small range of creatinine clearance in the study population. A relatively high exposure of moxifloxacin in patients with renal insufficiency could be underestimated as renal function may be overestimated and the other way around for patients with normal renal function and relatively low exposures. The pharmacokinetic modelling software will fit a curve with the greatest likelihood of being the actual pharmacokinetic curve based on drug concentrations at 0 and 6 h together with patient characteristics and data of the entire population. However, when influence of creatinine clearance is not available the software will pick a fit with average parameters, causing overestimation in low AUC<sub>0-24</sub> and underestimation in high AUC<sub>0-24</sub> ranges. We decided not to validate one of the better

222 performing three-sample strategies from Table 5, since we focussed on developing a clinically feasible  
223 LSS with a strong preference for only 2 samples. Furthermore, we aimed to provide a simple and well  
224 performing alternative LSS for MFX using multiple linear regression (t=0 and 4 h). We recommend to use  
225 this LSS instead of the Bayesian LSS for MFX, particularly when low drug exposure is suspected, because  
226 overestimation of  $AUC_{0-24}$  can lead to sub therapeutic dosing with treatment failure and acquired drug  
227 resistance as possible harmful consequence.(26, 36, 37)

228 In this study we decided to validate one LSS for each situation (Bayesian or multiple linear regression;  
229 MFX or MFX+RIF), due to the significant influence of rifampicin on the pharmacokinetics of moxifloxacin  
230 and so there would be a suitable LSS for every patient in each health care centre. The LSSs using multiple  
231 linear regression performed rather well in our study population, but is less flexible in patients with  
232 different characteristics. A Bayesian LSS is therefore preferred for patients who are not comparable to  
233 our study populations as the population pharmacokinetic model is able to include some patient  
234 characteristics. Clinicians are guided to the best option for TDM of moxifloxacin by following the decision  
235 tree in Figure 8. For implementation of moxifloxacin TDM using LSSs in daily practice, it would be  
236 convenient to be able to use one sampling strategy for both MFX and MFX+RIF. This study showed that it  
237 is possible to use t=0 and 6 h samples in a Bayesian LSS for both MFX as well as MFX+RIF and probably  
238 even in a multiple linear regression LSS for MFX+RIF after successful validation. Unfortunately, a multiple  
239 linear regression strategy for MFX alone using t=0 and 6 h samples was not feasible because of inferior  
240 performance. Considering that TB patients are treated with a combination of multiple anti-TB drugs, one  
241 single LSS suitable for all drugs of interest is the ideal situation, but unfortunately also rather challenging  
242 due to the various pharmacokinetic properties of the different drugs. Others did succeed in developing a  
243 LSS using multiple linear regression for simultaneously estimating exposure of all first-line drugs and  
244 moxifloxacin in a small population of TB patients.(20) A 2 and 6 h post-dose sampling strategy is  
245 frequently used for TDM of anti-TB drugs as it is believed to be able to estimate  $C_{max}$  as well as to detect

246 delayed absorption.(31) However, better performances were found for the LSSs proposed in this study,  
247 although the 2 and 6 h LSS performed within acceptable limits as well in the Bayesian approach and the  
248 multiple linear regression.

249 In general, we noticed large inter-individual pharmacokinetic variation in terms of moxifloxacin  
250 concentrations (Figure 1),  $C_{\max}$ , and  $AUC_{0-24}$  (Table 2) as described earlier,(18) but also in  $K_a$  and  $CL/F$   
251 (Table 4). Patients received 400, 600, or 800 mg moxifloxacin; this obviously influenced drug  
252 concentration,  $C_{\max}$ , and  $AUC_{0-24}$ , but not all variation could be explained by different dosage regimes. For  
253 MFX,  $AUC_{0-24}$  corrected to a 400 mg standard dose was ranged from 10.2 to 79.1 mg\*h/L and for  
254 MFX+RIF a range of 10.0 to 47.4 mg\*h/L. This substantial inter-individual variation is the reason why  
255 TDM of moxifloxacin is helpful to assure optimal drug exposure and thus minimize the risk of treatment  
256 failure and developing acquired drug resistance.(26, 27) The estimated  $AUC_{0-24}$  using one of the LSS  
257 proposed together with the MIC of the *M. tuberculosis* strain will provide valuable information on the  
258 optimal moxifloxacin dose to be used in an individual patient.

259 A limitation to the study is the exclusion of the creatinine clearance from the population  
260 pharmacokinetic model. As discussed earlier, this could have led to the observed bias in the MFX LSS  
261 using 0 and 6 h samples as approximately 20% of moxifloxacin is eliminated unchanged in the urine. On  
262 the contrary, a well performing LSS using multiple linear regression (t=0 and 4 h) is a suitable alternative  
263 for MFX. The lack of prospective or external validation of the population pharmacokinetic model and  
264 LSSs could be considered as another limitation. However, we were able to collect a large dataset to  
265 develop the model and clinically feasible LSSs using a sufficient number of pharmacokinetic profiles. A  
266 strength of our study was that a large part of our dataset consisted of drug concentrations which were  
267 collected as part of daily routine TDM. During visual check of the data we noticed several abnormal  
268 curves (both MFX and MFX+RIF) that for instance showed delayed absorption with  $T_{\max}$  values of 4-6 h.  
269 These curves were not excluded from the study. The models and LSSs appeared to be able to adapt to

270 this delayed absorption. In most cases, the subsequent decision to either increase the dose or not was  
271 similar. For these reasons, we expect the results as reported in this study to represent the clinical  
272 practice of TDM using these LSSs very closely. The small sample size of the MFX+RIF group can be  
273 considered as a limitation as well, although comparable to previously published LSS studies.(21, 38–41)  
274 We consider this sample size as sufficient for exploratory objectives, since this is the first study that  
275 developed separate LSSs for moxifloxacin alone and in combination with rifampicin. Future research can  
276 build on the results described in this study.  
277 In conclusion, we developed and validated two separate pharmacokinetic models for moxifloxacin alone  
278 and in combination with rifampicin in TB patients. We provided data to show significant differences in  
279 drug clearance and drug exposure between these groups. Furthermore, we developed and validated LSS  
280 based on the Bayesian approach (MFX and MFX+RIF 0 and 6 h) and multiple linear regression (MFX 0 and  
281 4 h; MFX+RIF 1 and 6 h) that can be used to perform TDM on moxifloxacin in TB patients.

282

## 283 **Materials and methods**

### 284 Study population

285 This study used three databases. Database 1 consisted of retrospective data of routine TDM in 67  
286 tuberculosis patients treated at Tuberculosis Center Beatrxoord, University Medical Center Groningen,  
287 The Netherlands and was collected between January 2006 and May 2017, partly published earlier.(18) All  
288 patients received moxifloxacin (with or without rifampicin) as part of their daily TB treatment and  
289 pharmacokinetic curves were obtained as part of routine TDM care. Each patient was only included once.  
290 Varying sampling schedules were used, but most profiles included t=0, and 1, 2, 3, 4, and 8 h post-dose  
291 samples. Pharmacokinetic profiles consisting of less than 3 data points were excluded. The second  
292 database included data of 25 TB patients participating in a clinical study in Thessaloniki, Greece.(33)  
293 After at least 12 days of treatment with moxifloxacin with or without rifampicin, blood samples were

294 collected at t=0, and 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after drug intake. The third database consisted of  
295 pharmacokinetic data of 9 Brazilian TB patients receiving 400 mg moxifloxacin (no rifampicin) daily in an  
296 early bactericidal activity study.(14) At the fifth day, blood samples were collected at t=0, and 1, 2, 4, 8,  
297 12, 18 and 24 h after drug intake.

298 As steady state is reached within 3-5 days of treatment with moxifloxacin, all data was collected during  
299 steady state conditions.(11) In general, no informed consent was required, due to the retrospective  
300 nature of the study.

301 The total study population was split in two groups; patients that received moxifloxacin alone (MFX) and  
302 patients that received moxifloxacin together with rifampicin (MFX+RIF), because of the pharmacokinetic  
303 drug-drug interaction between rifampicin and moxifloxacin.(10) As sample collection in the MFX+RIF  
304 group was performed after a median number of days on rifampicin treatment of 35 (IQR 13-87),  
305 maximum enzyme induction by rifampicin was expected to be reached in most patients.(35)

306 Patient characteristics of both groups were tested for significant differences, median (interquartile range  
307 (IQR)) using the Mann-Whitney U test and n (%) using the Fisher's exact test in IBM SPSS Statistics (23,  
308 IBM Corp., Armonk, NY). P values <0.05 were considered significant.

309

310 Population pharmacokinetic model

311 For each group, MFX and MFX+RIF, a population pharmacokinetic model was developed using the  
312 iterative two-stage Bayesian procedure of the KinPop module of MWPharm (version 3.82, Mediware,  
313 The Netherlands). As the pharmacokinetics of moxifloxacin have been described with one compartment  
314 (14, 21) as well as two-compartment models (42, 43), both types were evaluated. The population  
315 pharmacokinetic parameters of the models were assumed to be log normally distributed with a residual  
316 error and concentration dependent standard deviation ( $SD=0.1+0.1 \cdot C$ , where C is the moxifloxacin  
317 concentration in mg/L). Because the bioavailability (F) of moxifloxacin is almost complete (11) and

318 pharmacokinetic data following intravenous administration was not available, F was fixed at 1 in the  
319 analysis and pharmacokinetic parameters are presented relative to F. Moxifloxacin is mainly metabolised  
320 in the liver by glucuronosyltransferase and sulfotransferase (approximately 80%).(11) Only total body  
321 clearance (CL), the sum of metabolic and renal clearance, was included in the model development,  
322 because it was not possible to determine renal clearance due to a small range of creatinine clearance  
323 values in our dataset.

324 We started the analysis with a single default one compartment model for both MFX and MFX+RIF  
325 developed by Pranger *et al* using a very similar methodology.(21) This study found comparable  
326 pharmacokinetic parameters of MFX and MFX+RIF, although likely due to a small sample size. Two  
327 default two compartment models were used, one for MFX and one for MFX+RIF.(42, 44) Modelling was  
328 started with all parameters fixed and Akaike Information Criterion (AIC) was used to evaluate the  
329 model.(45) Subsequently, one by one parameters were Bayesian estimated and each step was evaluated  
330 by calculation of the AIC. A reduction of the AIC with at least 3 points was regarded as a significant  
331 improvement of the model.(46) One compartment models included the parameters CL, volume of  
332 distribution (V), and absorption rate constant ( $K_a$ ). Two compartment models included the parameters  
333  $K_a$ , CL, inter-compartmental clearance ( $CL_{12}$ ), central volume of distribution ( $V_1$ ), volume of distribution of  
334 the second compartment ( $V_2$ ), and lag time for absorption ( $T_{lag}$ ). Afterwards,  $T_{lag}$  was added to the best  
335 performing one compartment model and evaluated for goodness of fit as well, because of oral intake of  
336 moxifloxacin. The default two compartment models already included  $T_{lag}$ . The final models of MFX and  
337 MFX+RIF were chosen based on AIC values.

338 The final models were internally validated using 11 different (n-7) sub models for MFX and 12 (n-2) sub  
339 models for MFX+RIF, each leaving out randomly chosen pharmacokinetic curves. All pharmacokinetic  
340 curves were excluded once (jackknife analysis). The Bayesian fitted  $AUC_{0-24}$  of each left out curve ( $AUC_{0-24, fit}$ )  
341 was compared with the  $AUC_{0-24}$  calculated with the trapezoidal rule ( $AUC_{0-24, ref}$ ) using a Bland-Altman



342 plot and Passing Bablok regression (Analyse-it 4.81, Analyse-it Software Ltd, Leeds, United Kingdom). In  
343 the calculation of  $AUC_{0-24, ref}$ , moxifloxacin concentrations at  $t=0$  and 24 h after drug intake were assumed  
344 to be equal due to steady state conditions.  $C_{max}$  (mg/L) was defined as the highest observed moxifloxacin  
345 concentration and  $T_{max}$  (h) as the time at which  $C_{max}$  occurred. Non-compartmental parameters ( $AUC_{0-24,}$   
346  $_{ref}$ , dose-corrected  $AUC_{0-24, ref}$  to the standard dose of 400 mg,  $C_{max}$ ,  $T_{max}$ ) and population pharmacokinetic  
347 model parameters of the MFX and MFX+RIF group were compared and tested for significant differences  
348 using the Mann-Whitney U test.

349

350 LSS using Bayesian approach

351 Using the Bayesian approach, we performed two separate analyses to develop LSSs; one for MFX and  
352 one for MFX+RIF. Using Monte Carlo simulation in MWPharm, 1000 virtual pharmacokinetic profiles  
353 were created to represent the pharmacokinetic data used in the development of the LSS. The reference  
354 patient for the Monte Carlo simulation was selected based on representative pharmacokinetic data and  
355 patient characteristics. For MFX, a 36 year old male with a bodyweight of 57 kg, height of 1.60 m, BMI of  
356  $22.2 \text{ kg/m}^2$ , serum creatinine of  $74 \text{ } \mu\text{mol/L}$ , and moxifloxacin dose of  $7.0 \text{ mg/kg}$  was chosen. For  
357 MFX+RIF, a 56 year old male with a bodyweight of 56 kg, height of 1.63 m, BMI of  $21.1 \text{ kg/m}^2$ , serum  
358 creatinine of  $80 \text{ } \mu\text{mol/L}$ , and moxifloxacin dose of  $7.1 \text{ mg/kg}$  was selected. The LSSs were optimised using  
359 the steady state  $AUC_{0-24}$ . Only clinically feasible LSSs using 1-3 samples between 0 and 6 h post-dose and  
360 sample interval of 1 h were tested. The LSSs were evaluated using acceptance criteria for precision and  
361 bias ( $RMSE < 15\%$ ,  $MPE < 5\%$ ). (18) For both MFX and MFX+RIF, one LSS was chosen for internal validation  
362 based on performance as well as clinical feasibility. The  $AUC_{0-24}$  estimated with the chosen LSS ( $AUC_{0-24,}$   
363  $_{est}$ ) was compared with  $AUC_{0-24, ref}$  using a Bland-Altman plot and Passing Bablok regression. Additionally,  
364 the performance of a LSS using 2 and 6 h post-dose samples was evaluated, because this is a LSS  
365 frequently used for TDM of anti-TB drugs. (31)

366

367 LSS using multiple linear regression

368 Two separate analyses (MFX and MFX+RIF) using multiple linear regression were performed.

369 Only clinically suitable LSSs (1-3 samples, 0-6 h post-dose, sample interval 1 h) were included in the

370 analysis. Each analysis excluded the pharmacokinetic curves without data at the selected time points of

371 the LSS, resulting in a variable number of included curves (N). Multiple linear regression in Microsoft

372 Office Excel 2010 was used to evaluate the correlation of moxifloxacin concentrations at the chosen time

373 points of the LSS and  $AUC_{0-24, \text{ref}}$ . The acceptance criteria (RMSE<15%, MPE<5%) were applied to each

374 LSS.(18) Internal validation using 11 different (n-6) sub analyses for MFX and 14 (n-1) sub analyses for

375 MFX+RIF was used to evaluate the performance of the LSSs. Each sub analysis excluded randomly chosen

376 profiles and all profiles were excluded once (jackknife analysis). Agreement of  $AUC_{0-24, \text{est}}$  and  $AUC_{0-24, \text{ref}}$ 

377 was tested using a Bland-Altman plot and Passing Bablok regression.

378

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512

513 Table 1. Patient characteristics of the study population. Data is presented as median (IQR) unless  
514 otherwise stated.

Parameter	MFX n=77	MFX+RIF n=24	P value
Male sex [n(%)]	47 (61.0)	21 (87.5)	0.023 <sup>a</sup>
Age (yr)	33 (25-41)	48 (36-62)	<0.001 <sup>b</sup>
Ht (m)	1.65 (1.59-1.74)	1.72 (1.64-1.76)	0.047 <sup>b</sup>
Wt (kg)	58.0 (52.5-68.2)	55.5 (52.3-63.9)	0.500 <sup>b</sup>
Dose (mg/kg bodywt)	7.0 (5.9-8.1)	7.3 (6.4-7.7)	0.629 <sup>b</sup>
BMI (kg/m <sup>2</sup> )	21.2 (19.3-23.5)	20.1 (17.6-22.7)	0.053 <sup>b</sup>
Serum creatinine (μmol/L)	71 (59-83)	73 (63-91)	0.752 <sup>b</sup>
Number of samples per curve	7 (6-8)	10 (7-10)	<0.001 <sup>b</sup>
Days on rifampicin treatment at time of sampling	NA	35 (13-87)	NA

515 <sup>a</sup> Fisher exact test

516 <sup>b</sup> Mann-Whitney U test

517

- 518 Table 2. Non-compartmental parameters ( $AUC_{0-24, \text{ref}}$ , dose corrected  $AUC_{0-24, \text{ref}}$  to 400 mg standard dose,  
519  $C_{\text{max}}$  and  $T_{\text{max}}$ ) of MFX and MFX+RIF, presented as median (IQR).

Parameter	MFX (n=77)	MFX+RIF (n=24)	P-value
$AUC_{0-24, \text{ref}}$ (mg·h/L)	34.0 (25.2-49.2)	25.5 (20.4-31.6)	0.006 <sup>a</sup>
Dose corrected $AUC_{0-24, \text{ref}}$ (mg·h/L, per 400 mg)	30.8 (24.7-40.3)	25.5 (19.1-31.3)	0.014 <sup>a</sup>
$C_{\text{max}}$ (mg/L)	3.00 (2.27-4.64)	2.83 (2.25-3.90)	0.407 <sup>a</sup>
$T_{\text{max}}$ (h)	2 (1-3)	1.5 (1-2)	0.018 <sup>a</sup>

- 520 <sup>a</sup> Mann-Whitney U test

- 521 Table 3. Starting parameters of the default one compartment and two compartment models of MFX and  
522 MFX+RIF together with the parameters of the final models based on AIC.

Parameter	Default model MFX	Final model MFX	Default model MFX+RIF	Final model MFX+RIF
One compartment				
CL (L/h)	18.500±8.600	14.655±5.683	18.500±8.600	19.898±8.800
$V_d$ (L/kg bodyweight)	3.000±0.7000	2.7467±1.0077	3.000±0.7000	2.8264±0.6902
$K_a$ (/h)	1.1500±1.1600	6.2904±4.8164	1.1500±1.1600	7.3755±6.8205
$T_{\text{lag}}$ (h)	NA	0.8769±0.2357	NA	0.7460±0.1093
AIC	5564	903	1361	236
Two compartments				
CL (L/h)	11.800±0.740	13.428±5.494	49.100±2.550	18.108±8.570
$CL_{12}$ (L/h)	5.620±1.080	5.620±1.080	3.150±0.800	3.150±0.800
$V_1$ (L/kg bodyweight)	2.5300±0.0800	2.4898±1.0838	2.8400±0.1500	2.7004±0.7535

$V_d$ (L/kg bodyweight)	0.6900±0.1300	0.6900±0.1300	0.8900±0.1900	0.8900±0.1900
$K_a$ (/h)	16.7000±2.9200	3.2774±2.9422	2.3200±0.5600	6.2314±9.0508
$T_{lag}$ (h)	0.4600±0.0800	0.7940±0.3720	0.6000±0.0700	0.7312±0.1995
AIC	11892	940	2995	249

523

524 Table 4. Comparison of pharmacokinetic parameters of the population pharmacokinetic model of MFX

525 versus MFX+RIF. Geometric mean±SD.

Parameter	MFX (n=77)	MFX+RIF (n=24)	P value
CL/F (L/h)	14.655±5.683	19.898±8.800	0.004 <sup>a</sup>
$V_d$ /F (L/kg bodyweight)	2.7467±1.0077	2.8264±0.6902	0.534 <sup>a</sup>
$K_a$ (/h)	6.2904±4.8164	7.3755±6.8205	0.231 <sup>a</sup>
$T_{lag}$ (h)	0.8769±0.2357	0.7460±0.1093	<0.001 <sup>a</sup>

526 <sup>a</sup> Mann-Whitney U test

527

528 Table 5. LSSs of moxifloxacin without RIF using the Bayesian approach, including MPE, RMSE, and  $r^2$ .

Sampling time point (h)			MPE (%)	RMSE (%)	$r^2$
5			2.69	24.64	0.659
6			1.74	22.00	0.726
2	6		-2.20	20.83	0.742
0	5		2.84	15.82	0.864
0	6		2.42	15.17	0.874
0	4	6	0.97	13.22	0.883

0	5	6	1.03	12.97	0.888
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529

530 Table 6. LSSs of moxifloxacin with RIF using the Bayesian approach, including MPE, RMSE, and  $r^2$ .

Sampling time point (h)			MPE (%)	RMSE (%)	$r^2$
5			-1.97	22.35	0.768
6			-0.79	19.22	0.826
2	6		-2.89	18.38	0.832
0	5		1.88	16.67	0.877
0	6		2.35	15.81	0.885
0	4	6	1.06	14.10	0.907
0	5	6	0.79	13.73	0.912

531

532 Table 7. LSSs of moxifloxacin without RIF using linear regression, including the equation to calculate

533  $AUC_{0-24, est}$ , number of included curves (N), MPE, RMSE, and  $r^2$ .

Sampling time point (h)			Equation <sup>a</sup>	N	MPE (%)	RMSE (%)	$r^2$
4			$AUC_{0-24, est} = 3.47 + 12.32 * C4$	66	12.68	17.02	0.862
6			$AUC_{0-24, est} = 2.27 + 15.01 * C6$	22	14.85	16.89	0.822
2	6		$AUC_{0-24, est} = -1.44 + 3.55 * C2 + 11.24 * C6$	22	10.02	12.27	0.901
0	3		$AUC_{0-24, est} = 3.61 + 28.67 * C0 + 5.38 * C3$	53	10.08	13.36	0.917
0	4		$AUC_{0-24, est} = 1.10 + 20.76 * C0 + 8.68 * C4$	66	6.85	9.42	0.957

0	2	4	$AUC_{0-24, est} = 1.10 + 20.37 * C_0 + 0.92 * C_2 + 7.71 * C_4$	65	6.91	9.25	0.958
0	1	4	$AUC_{0-24, est} = 1.00 + 21.06 * C_0 + 0.66 * C_1 + 8.02 * C_4$	63	7.07	9.23	0.958

<sup>a</sup> C<sub>0</sub>, C<sub>1</sub>, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

Table 8. LSSs of MFX+RIF using multiple linear regression, including the equation to calculate  $AUC_{0-24, est}$

number of included curves (N), MPE, RMSE, and  $r^2$ .

Sampling time point (h)			Equation <sup>a</sup>	N	MPE (%)	RMSE (%)	$r^2$
3			$AUC_{0-24, est} = -2.76 + 13.28 * C_3$	18	8.27	11.10	0.907
6			$AUC_{0-24, est} = 0.95 + 16.44 * C_6$	16	6.93	8.87	0.941
2	6		$AUC_{0-24, est} = 0.08 + 1.21 * C_2 + 15.02 * C_6$	13	6.23	7.88	0.945
0	6		$AUC_{0-24, est} = 1.38 + 7.40 * C_0 + 14.05 * C_6$	16	5.85	6.99	0.960
1	6		$AUC_{0-24, est} = 1.43 + 0.22 * C_1 + 16.25 * C_6$	14	4.83	6.09	0.971
0	3	6	$AUC_{0-24, est} = 1.20 + 10.66 * C_0 - 0.39 * C_3 + 13.52 * C_6$	15	4.85	5.31	0.977
0	2	6	$AUC_{0-24, est} = 0.46 + 9.99 * C_0 + 0.13 * C_2 + 13.39 * C_6$	13	4.20	4.66	0.978

<sup>a</sup> C<sub>0</sub>, C<sub>1</sub>, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

539 Figure 1. Moxifloxacin concentrations of the pharmacokinetic curves of MFX (n=77) and MFX+RIF (n=24)

540

541 Figure 2. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n=7) of

542 population pharmacokinetic model of MFX (n=77).

543

544 Figure 3. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n=2) of

545 population pharmacokinetic model of MFX+RIF (n=24).

546

547 Figure 4. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS

548 (t=0 and 6 h) of MFX (n=77).

549

550 Figure 5. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS

551 (t=0 and 6 h) of MFX+RIF (n=24).

552

553 Figure 6. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n=6) of LSS using

554 multiple linear regression (t=0 and 4 h) of MFX (n=66).

555

556 Figure 7. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n=1) of LSS using

557 multiple linear regression (t=1 and 6 h) of MFX+RIF (n=14).

558

559 Figure 8. Clinical guide for choosing the best LSS for TDM of moxifloxacin alone or in combination with  
560 rifampicin.

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